Design, synthesis and pharmacological evaluation of Enone prodrugs

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Chapter 6

Mono-pivaloyl esters of apomorphine: New prodrugs with anti-Parkinsonian potential

Abstract

Apomorphine is a dopamine (DA) agonist nowadays used for the treatment of on-off symptoms in the advanced stage of Parkinson's disease (PD). However, this compound has poor pharmacokinetic properties when given orally. A classical way to try to enhance its action *in vivo* is esterification. In this chapter the synthesis of *mono*-pivaloyl esters of apomorphine (**6.2**/**6.3**) is described.

The pharmacological effects of **6.2**/**6.3** were studied by measuring their effects on extracellular DA levels in the corpus striatum, the brain area of interest in PD, using microdialysis in freely moving rats. To have an indication of the bioavailability of these prodrugs, plasma levels of apomorphine were measured after *sc* application of apomorphine and the prodrug, respectively. We have found that apomorphine produced high plasma peak levels that rapidly (within 2 h) declined to low plasma levels (<20 nM), whereas **6.2**/**6.3** showed lower plasma levels but maintained relatively high apomorphine levels (20-100 nM) during at least 6 h. The pharmacokinetic properties of the prodrugs were further improved when the compounds are administered in an oil suspension. It is concluded that the *mono*-pivaloyl esters of apomorphine might be considered for clinical treatment of PD.

This chapter is based on the work of Danyang Liu, Ulrike Dijkman, Ulrik Jurva, Jan de Vries, Durk Dijkstra and Håkan Wikström in Groningen University.

6.1 Introduction

Dopamine (DA) agonists are used as an alternative, or a complementary treatment to *L*-dopa in patients suffering from Parkinson's disease (PD). Examples are bromocriptine (**1.58**),[1] pergolide (**1.60**), [2] ropinirole (**1.63**),[3] pramipexole (**1.64**)[4], *etc*.[5] DA agonists are effective in patients in the advanced stages of PD when *L*-dopa is losing efficacy.

Apomorphine (**1.23**) is a DA D_1/D_2 agonist and was first synthesized from morphine in 1869. The main drawback of the clinical use of apomorphine (**1.23**), is its weak oral activity and short duration of action.[6] Oral administration of apomorphine tablets required high doses to achieve the necessary therapeutic effect. Such doses cause severe side-effects, such as sedation, respiratory depression, hypotension, bradycardia, sweating, yawning and emesis.[7] High oral doses of apomorphine induce hepatoxicity.

Because of the limitations of the oral administration, many alternative routes of administration have been studied including intranasal,[8] rectal,[9] sublingual[10] and transdermal.[11] The long-term use of sublingual apomorphine is also limited by the occurrence of stomatitis and mucosal ulceration in 50% of the treated patients.[7] Intranasal administration produced transient nasal blockage, burning sensation, and swollen nose and lips.[12] In some of the patients tested, intranasal administration had to be withdrawn because of inflammation of the nasal mucosa.[13]

So far, the most feasible clinical way of administering apomorphine, avoiding strong first pass metabolism, is subcutaneous administration. Apomorphine has been proven to be very effective in PD. Subcutaneously administered (R)-(−)-apomorphine in combination with *L*-dopa rapidly and consistently reverse the "off" period motor deficits.[6] Besides its action as a DA agonist, (R)-(−)-apomorphine can also act as radical scavenger and, therefore, may have neuroprotective properties. One of the major limitations however, is its low bioavailability. Because PD requires long term treatment, its use is limited since the injection site must be changed every 12 h to minimize risks of skin discoloration and nodule formation. Furthermore, subcutaneous administration does not avoid the normal DA agonist side effects, such as nausea and vomiting.[14]

It is apparent from the above that it would be highly desirable from a clinical point of view to find a way of administering apomorphine, with a longer duration of action.[15,16,17] Aporphine prodrugs, with enhanced chemical stability,[18,19,20] have been described and tested in animal models in the past.[21] Such prodrugs are often ester derivatives, *i.e.* 10,11-di-esters. For instance, the following di-esters of aporphines have been described: di-acetyl, di-propionyl, dibutyryl, di-iso-butyryl, di-pivaloyl, di-pentanoyl, di-hexanoyl, di-hexadecanoyl, di-phenylacetyl, di-methoxyacetyl, di-trifluoroacetyl and

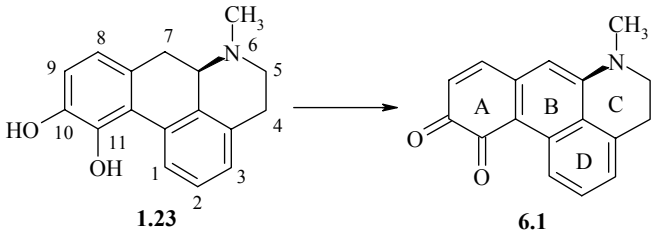
di-heptafluorobutanoyl esters.[22]

Improved bioavailability and longer duration of action have been published for some of these esters.[23, 24] The increased duration of action of the larger di-esters of apomorphine reflects probably the lipophilic properties of these compounds. A decreased rate of the hydrolysis is due to the increased steric hindrance around the acyl carbonyl center. As an example, the di-pivaloyl ester prodrug was much less active than the parent compound apomorphine itself, probably because of a decreased rate of hydrolysis due to the steric hindrance of the pivaloyl group.[22] The possibilities of preparing asymmetrical di-esters have been mentioned in US-A-4080456.[25] Because of the difficulties to prepare these asymmetrical di-esters, no further publications about these compounds have been appeared in the literature. In addition, the pharmacology of such di-esters is difficult to predict. Therefore all known di-ester of apomorphine, actually prepared, are symmetrically substituted.

The authors Borgman and Baldessarini found a slight increase in duration of action for the small esters of apomorphine, like di-acetyl ester and di-propionyl ester.[22] The di-pivaloyl and di-benzoyl esters produced prolonged stereotyped behaviour after giving large dose (50 μmol/kg i.p.).[23] However, Low stereotypic and turning activity was found when lower dose (2 μmol/kg) di-pivaloyl apomorphine ester was given *sc* in reserpinized rats models.[26] The high dose of these esters is not preferable because of the increasing side effects. There is a need for improved ways of administration for apomorphine and/or apomorphine prodrugs, which are not well absorbed orally and/or are targets for extensive first pass elimination. Alternatively, these drugs may be applied to a patch for transdermal administration in a suitable composition and with a suitable vehicle (e.g. a penetration enhancer).

6.2 Chemistry

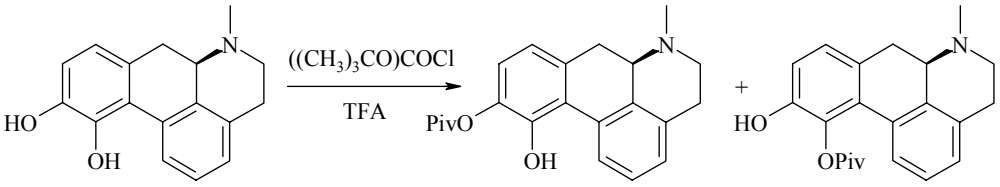
Apomorphine is very sensitive to oxidation, especially under basic conditions due to the catechol moiety.[27] The aromatization of ring B could be the driving force for this oxidation. It is thus virtually impossible to handle apomorphine in the free base form, without the addition of antioxidants.



1.23 **6.1**

Scheme 6.1 *The oxidation of apomorphine.*

The esterification (Scheme 6.2) of apomorphine was performed under acidic conditions in trifluoroacetic acid,[28] and pivaloyl chloride dissolved in CH_2Cl_2 was added to the apomorphine solution. The mono-pivaloyl ester of apomorphine is designed to be easily hydrolyzed, making it difficult to use SiO_2 and nucleophilic alcohols during purification with column chromatography. Purification was performed by column chromatography on Al_2O_3. Unreacted apomorphine, like all catechols, sticks to the column. The synthesis of mono-pivaloylapomorphine gave a mixture of regio-isomers. NMR showed a 75% fraction which was believed to be 10-pivaloyl-apomorhine (**6.2**) and 25% of the 11-isomer (**6.3**). We did not succeed in separating these regio-isomers.



1.23 **6.2** **6.3**

Scheme 6.2 *Preparation of mono-pivaloyl apomorphine. Reagents and conditions: $((CH_3)_3CO)COCl$, CF_3COOH, CH_2Cl_2, $0^oC \rightarrow RT$, overnight.*

6.3 Pharmacology

6.3.1 *In vivo* pharmacology

The potential pharmacological effects of the mixture of regio-isomers **6.2/6.3** were studied by measuring their effects on extracellular DA levels in the corpus striatum, the brain area of interest in PD, using microdialysis in freely moving rats.[29] Details see Chapter 2.

6.3.2 The metabolites analysis of plasma samples

The main aim of these studies was to provide further evidence for the *in vivo* hydrolyzation of mono-pivaloyl apomorphine ester (**6.2/6.3**). After the administration *sc* of these esters, the plasma concentration of produced apomorphine (**1.23**), which is the active component *in vivo*, was detected in 6 h. As comparison, the plasma concentration of apomorphine was detected after the administration *sc* of apomorphine itself. The different dissolvents, such as saline and viscoleo, were expected to have different affects on the hydrolyzation of these esters.

6.4 Results and discussion

6.4.1 Chemistry

The mixture of the two *mono*-pivaloyl-apomorphine isomers was synthesized in the presence of trifluoroacetic acid and the reaction was performed at RT. The reaction was easy to handle, however, the yield was poor (21.8%). Probably during the purification of the raw product *via* column chromatography, partial hydrolysis of the products occurred. Hydrolysis of the product was enhanced by acidic silica gel, and therefore, neutral aluminum oxide was used. Reasonably, the 10-position is more accessible for ester formation, because of the phenyl moiety that impedes esterification of the 11-position. GC on the acetylated mixture showed two peaks, which are the two mixed di-esters: 10-pivaloyl, 11-acetyl and 10-acetyl, 11-pivaloyl esters. Just a small amount fraction of the di-pivaloyl ester of apomorphine was formed. This di-ester was removed by crystallization.

6.4.2 Pharmacology

6.4.2.1 Microdialysis study

6.2/**6.3** was administered *sc* to male Wistar rats at different doses (0.1 μmol, 1 μmol and 10 μmol/kg). Figure 6.1 shows that *sc* administration of **6.2**/**6.3** induced a dose-dependent decrease in the release of DA in the striatum.

A maximal 40% DA decrease of basal levels was found with 0.1 μmol kg^{-1} *sc* injection 45 min after administration. After 150 min, DA levels returned to basal levels.

Administration of 1 µmol/kg *sc* induced a significant effect, which lasted from 15 min until 250 min post administration ($p<0.05$). A maximal decrease of DA to 30% of basal levels was observed. After 4 h, the DA release returned to basal levels. After 10 µmol/kg *sc* administration, a decrease of DA levels was found after 15 min ($p<0.05$). The DA decrease remained significant from basal levels during the 6 h lasting experiments.

After *sc* administration of 1 and 10 µmol/kg, **6.2**/**6.3** induced locomotor activities, like penile licking, grooming, yawning, sniffing and rearing, which are consistent with the post-synaptic effects of a centrally acting DA agonist.

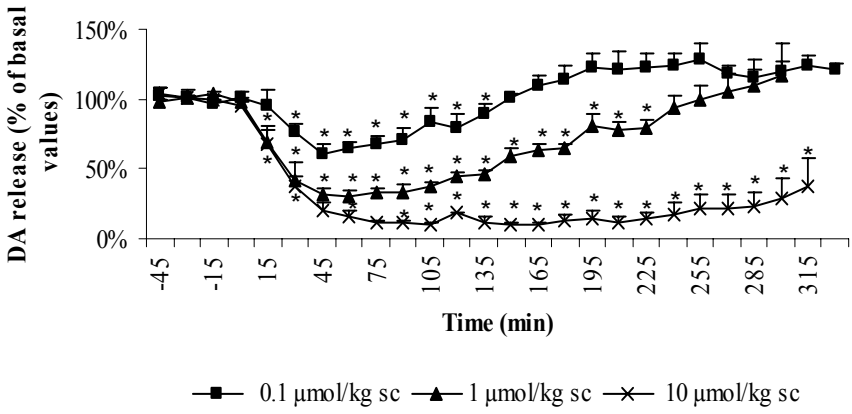


Figure 6.1 *Effect of* ***6.2***/***6.3*** *(0.1, 1, 10 µmol/kg sc, ■, ▲, x respectively.) on striatal DA release in freely moving rats. The results are the mean (± SEM) of data obtained from 4 rats (* $p<0.05$).*

6.4.2.2 Plasma levels of apomorphine and its pivaloyl ester prodrug

Apomorphine (**1.23**, 4.0 mg/kg, 13.2 µmol/kg) dissolved in saline with the help of a drop of acetic acid was given *sc* to rat. After 15 min the plasma concentration was about 1000 nmol/L. After 50 min, the plasma concentration decreased to 100 nmol/L. After 5 h, it reached 5 nmol/L (Figure 6.2).

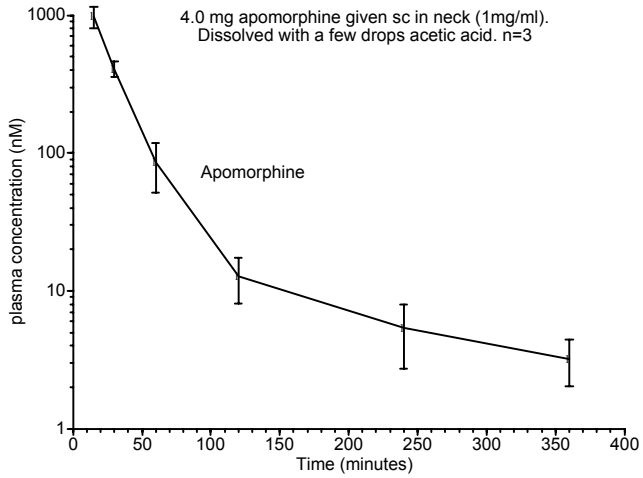


Figure 6.2 *Apomorphine plasma concentration after sc administration.*

Mono-pivaloyl apomorphine (**6.2**/**6.3**, 4.6 mg/kg, 13.2 μmol/kg *sc*), dissolved in saline with the help of a drop of acetic acid, was given to rats. The initial plasma concentration of the prodrug after 15 min was 600 nmol/L (Figure 6.3). The plasma level decreased almost linearly and even after 5 h, the plasma concentration of the monopivaloyl ester is decreased to 20 nmol/L. After administration of **6.2**/**6.3**, the produced plasma concentration of apomorphine was approximately 150 nmol/L and the curve was rather flat for 2 h. During the next 2 h the concentration decreased to 70 nmol/L. After 6 h, the concentration of apomorphine was still more than 20 nmol/L.

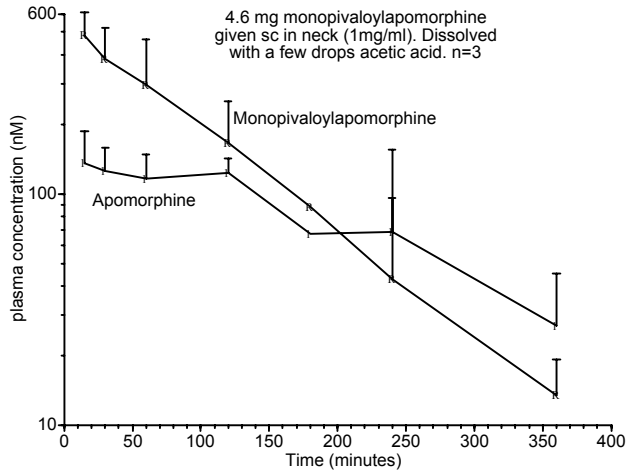


Figure 6.3 *The produced apomorphine plasma concentration after sc administration of mono-pivaloyl apomorphine dissolved in diluted acetic acid.*

It is evident that when equimolar amounts of apomorphine (**1.23**) and the

mono-pivaloyl prodrug (**6.2**/**6.3**) are compared with respect to apomorphine blood levels, apomorphine itself rapidly reaches high plasma peak levels that decrease to low levels (<10 nM) within 2 h, whereas the prodrug produced lower apomorphine peak levels but a relatively high plasma concentration (20-100 nM) of apomorphine was maintained for several (>6) h. Therefore, it is concluded that *mono*-pivaloyl apomorphine has a much longer duration of action than apomorphine itself.

In another experiment, *mono*-pivaloyl apomorphine (**6.2**/**6.3**, 4.6 mg/kg, 13.2 µmol/kg) was dissolved in viscoleo and this oil suspension was given to rat *sc* (Figure 6.4). *Mono*-pivaloyl apomorphine (**6.2**/**6.3**) was hydrolyzed to apomorphine immediately after injection. After 1 h, the produced apomorphine concentration reached the maximum level of 150 nmol/L, and it decreased to 80 nmol/L after 6 h. The long duration of action after the viscoleo injection was obvious, since after 20 h the apomorphine concentration is still higher than 20 nmol/L.

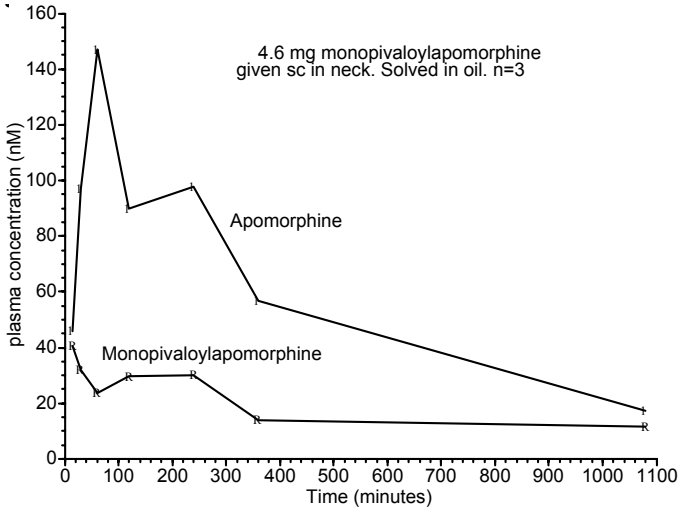


Figure 6.4 *Effect of sc injection of 4.6 mg/kg* ***6.2/6.3*** *dissolved in viscoleo on the prodrug itself and the produced apomorphine.*

Comparing the *sc* injection of both oil suspension and saline solution of *mono*-pivaloyl apomorphine (**6.2**/**6.3**), it is evident that administration in oil led to a similar hydrolysis and initial apomorphine concentrations (150 nmol/L), comparing with the released product in saline. After 5 h, the plasma level of apomorphine after administration of the oil suspension was still higher that 80 nmol/L, when the prodrug was dissolved in saline this concentration was kept only for 2.5 h. Furthermore, the plasma level of apomorphine after administration of the oil suspension was higher than 20 nmol/L for more than 20 h,

however, in saline it was only 6 h.

Therefore, *mono*-pivaloyl apomorphine (**6.2**/**6.3**) dissolved in oil has a much longer duration of action compared to in saline.

Figure 6.5 shows the effect of a low dose of *mono*-pivaloyl apomorphine (**6.2**/**6.3**, 2.3 mg/kg, 6.6 µmol/kg) dissolved in viscoleo injection. For comparison, apomorphine freebase (1.77 mg/kg, 6.6 µmol/kg) in viscoleo and apomorphine salt in saline (**1.23**) (2 mg/kg, 6.6 µmol/kg) were also tested. The *mono*-pivaloyl apomorphine (**6.2**/**6.3**) injection reached its highest apomorphine plasma concentration 50 nmol/L after 150 min, and the concentration decreased slowly, until 10 h, it decreased below 20 nmol/L. After the injection of apomorphine freebase in oil, the apomorphine plasma concentration reached to 100 nmol/L after 100 min, and during 5 h the concentration decreased to 20 nmol. The highest apomorphine plasma concentration (500 nmol/L) was reached immediately after the injection of apomorphine salt in saline. After 3 h this plasma concentration was decreased to 20 nmol/L.

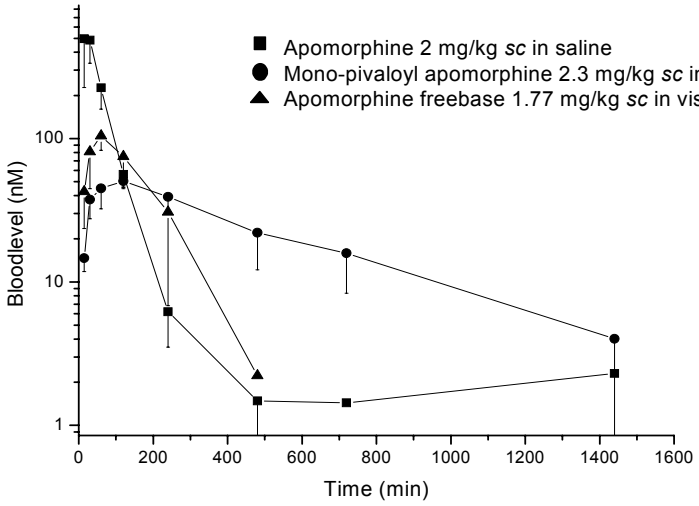


Figure 6.5 *Effect of 2.3 mg/kg mono-pivaloyl apomorphine dissolved in viscoleo, apomorphine 2 mg/kg sc in saline and 1.77 mg/kg sc in viscoleo.*

Comparing the *sc* injection of **6.2/6.3** in the dose of 4.6 mg/kg in viscoleo (Figure 6.4) with the half amount dose (2.3 mg/kg) (Figure 6.5), the initial plasma level of apomorphine from former (150 nM) was 3 times higher than latter (50 nM). After *sc* adminsitration of 4.6 mg/kg dose of **6.2/6.3** 12 h, the plasma level of apomorphine decreased to 20 nmol/kg, and it was 6 h for the same decrease of latter (2.3 mg/kg *sc* injection).

6.5 Conclusions

In conclusion, comparing equimolar *sc* injections of the *mono*-pivaloyl ester of apomorphine (**6.2**/**6.3**) and apomorphine itself, the plasma peak values of apomorphine induced by the parent compound are significantly higher than those produced by the prodrug. However, after the initial peak, apomorphine levels produced by the prodrug are maintained for several h at a higher concentration compared with the plasma levels of apomorphine itself. Avoiding high peak levels of apomorphine would be beneficial for preventing of adverse effects e.g. nausea, hallucinations, dyskinesia *etc*. Our observation points to a longer duration of action of the prodrug. The pharmacokinetic properties of the prodrug are further improved when the mixture (**6.2**/**6.3**) is administered in an oil suspension, as higher and longer lasting levels of apomorphine were produced when compared to injections in saline.

6.6 Experimental section

6.6.1 Chemistry

***Mono*-pivaloyl apomorphine**. Apomorphine**.**HCl (**1.23**, 0.45 g, 1.49 mmol) was dissolved in the mixture of CH_2Cl_2 (25 mL) and trifluoro-acetic acid (2 mL). The reaction was stirred on ice and pivaloyl chloride (0.54 g, 4.46 mmol) was added. The temperature slowly increased to RT and the reaction mixture was left overnight. The volatiles were removed *in vacuo*, yielding an yellow oil. Purification by column chromatography (Al_2O_3 natural, CH_2Cl_2:EtOH = 20:1) yielded the product as a colorless oil. Crystallization from *n*-hexane yielded gray crystal (114 mg, 21.8 %): mp 128-130°C; API-MS: m/z 351 (M^+). NMR showed a 75% fraction which was believed as 10-position pivaloyl-apomorphine and 25 % fraction as 11-position pivaloyl-apomorphine. ^{1}H-NMR 8.03 (0.6 H, J = 8.1 Hz), 7.64 (0.2 H, J = 7.7 Hz), 6.7-7.2 (m, 5H), 1.43-3.23 (m, 6H), 2.53 (s, 3H), 1.36 (s, 6.75H), 1.29 (s, 2.25 H) ppm.

6.6.2 Pharmacology

Animals. The test animals used were male rats of a Wistar derived strain (Harlan, the Netherlands) weighing 300-350 g. The rats were placed in a room with controlled environmental conditions (21^{o}C, humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). Food and water were ad libition available. Animals were not used during the first week after

arrival in the laboratory. Animal procedures were conducted in accordance with guidelines published in the NIH guide for the care and use of laboratory animals and all protocols were approved by the Groningen University Institutional Animal Care and use Committee.

Drugs. All drugs were tested as their hydrochloride salts unless noted otherwise. The drugs were dissolved in physiological (0.9%) saline immediately before use. All *in vivo* experiments were performed at the Animal Laboratory Unit of the University of Groningen, The Netherlands.

Surgery and brain microdialysis. On-line brain microdialysis in freely moving animals has previously been described.[30] Details see Chapter 2.

Sampling and extraction of blood. A permanent cannula was placed in the vena jugularis. After the surgery the rats were given at least 24 h to recover. Blood was taken from the cannula with an injection syringe and PE-tubing with the diameter of 0.75 mm. The sampling times were: t = 0, 15, 30, 60, 120, 240, 480, 720 and 1440 min. 0.35 mL of blood was collected in the Eppendorf test tubes to which 10 µL of 0.35 % mercaptoethanol and 10 µL of 10% EDTA was added (the final concentration mercapto ethanol is 0.01 %). The blood was centrifuged for five minutes at 4^{o}C and 3500 rpm. 200 µL of plasma was pipetted off and transferred into clean test tubes which were stored at -18°C until analysis.

Extraction of plasma. To the plasma samples a solution containing 500 ng/mL NPA (*N*-propyl-nor-apomorphine) was added. NPA is the internal standard with a final concentration of 50 ng/mL. Next, 100 µL of a 1 % solution of sodium hydrogen carbonate ($NaHCO_3$) and 3 mL of diethyl ether was added and the test tube was shaken for three minutes on a Multivortex shaker followed by centrifugation during 15 min at 4°C and 1000 rpm. The ether layer was pipetted off and transferred to another test tube. The ether extraction was performed three times and the ether layer was evaporated with warm water and nitrogen gas flowing over the surface. The remains in the test tubes were dissolved in 100 µL of the mobile phase and injected to analysis on the HPLC system.

Apparatuses. The samples were analyzed with an RP-HPLC with an electrochemical detector. The system consisted of an ANTEC electrochemical detector, a C-18 column, a GILSON 231 sampler injector and a GILSON 401 Diluter, a PHARMACIA HPLC pump 2150, and Kipp en Zonen flatbed recorder. The flow rate was 0.25 mL/min.

The mobile phase was: 2000 mL UP (ultra filtered) water, 860 mL methanol, 34 g citric acid monohydrate, 13.5 g $NaHPO_3.2H_2O$, 1.43 g EDTA, 25 mg/L OSA,1 mM TMA.

6.7 References

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